Contributions to Entomology 75 (1) 2025, 191–202 | DOI 10.3897/contrib.entomol.75.e139899

CONTRIBUTIONS TO ENTOMOLOGY

SENCKENBERG

Research Article

Green synthesized silver nanoparticles using *Trachyspermum ammi* (TA-AgNPs): A potential bioinsecticide against mosquito stages

Vareesh Baghela[1], Kiran Meena[1], Muskan Kachhwaha[2], Rajbala Verma[1], Neetu Kachhwaha[1]

1 *Department of Zoology, University of Rajasthan, JLN Marg, Jaipur -302004, Rajasthan, India*

2 *Department of Economics, University of Rajasthan, JLN Marg, Jaipur -302004, Rajasthan, India*

https://zoobank.org/4829B1F9-A31A-4C75-B1D0-36A453458C57

Corresponding author: Neetu Kachhwaha (drneetu2011@gmail.com)

Academic editor: Mehmet Yaran | Received 21 October 2024 | Accepted 13 February 2025 | Published 24 March 2025

Abstract

Nanoparticles synthesized by chemical and physical methods use toxic reducing agents and expensive equipment. This study's objective is to create silver nanoparticles through an economical and environmentally-friendly green synthesis method, employing *Tachyspermum ammi* leaf extract as a capping and reducing agent. The green synthesized silver nanoparticles (TA-AgNPs) were characterized by UV/Visible spectrum (absorbance peak-419 nm), Scanning electron microscopy (15–45 nm), Energy dispersive X-ray analysis (peak at 3 keV), X-ray diffraction (crystalline nature), and FTIR (strong peak at 3122.96 cm^{-1}). Further, the potential of the synthesized silver nanoparticles was subjected against third and fourth larval instar and adult stages of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* mosquitoes. The entomological assays were conducted by following WHO guidelines (2005, 2022) at the dose concentrations of 0.5 ppm, 5 ppm, 15 ppm, 25 ppm, 35 ppm, 45 ppm (larvicidal), and 5 ppm, 20 ppm, 35 ppm, 55 ppm, 75 ppm, 95 ppm concentrations (adulticidal) of crude extract and TA-AgNPs. After 24 hours of exposure, the TA-AgNPs treated a group of *An. stephensi* larvae and adults showed 100% death at their highest dosage. TA-AgNPs demonstrated considerable and superior larvicidal and adulticidal action compared to crude extract. A one-way ANOVA with a $p < 0.05$ yielded highly significant findings across all genera. The values at the data's LC_{50}, LC_{90}, and LC_{95} endpoints were estimated using the probit plane regression analysis. Compared to *Ae. aegypti* and *Cx. quinquefasciatus*, the *An. stephensi* had the highest acute toxicity. TA-AgNPs offered a potent insecticide for limiting epidemics of mosquito-borne diseases.

Key Words

LC_{50}, Mosquito, probit regression, silver nanoparticles, *Trachyspermum ammi*

Introduction

Nanotechnology has revolutionized traditional research in biomedicine, therapeutics, diagnostics, bioengineering, biophysics, optics, and pharmacogenomics owing to its wide range of applications (Medina-Pérez et al. 2019). It has become a prominent field in which researchers are exploring the potential of nanoparticles to usher out their industrial, medical, commercial, and agricultural uses. Nanoparticles have a size range of 10^{-9} meter which imparts them molecular characteristics which have unique chemical and physical properties. Traditionally, nanoparticles are synthesized in the laboratory using chemical methods such as chemical reduction (Suriati et al. 2014), co-precipitation (Mascolo et al. 2013), chemical decomposition (Jiang et al. 2015), electrochemical synthesis (Johans et al. 2002) and photochemical synthesis (McGilvray et al. 2006). Physical methods of nanoparticle synthesis include pulse ablation (Khashan et al. 2016), sputtering fabrication (Wender et al. 2013), irradiations (Cong et al. 2018), and lithography fabrication (Colson et al. 2013). However, traditional methods of nanoparticle synthesis have some

shortcomings. Chemical methods use hazardous chemicals, have a long processing time, and involve the formation of unwanted by-products that are toxic to the environment. Physical methods require high temperatures, very expensive equipment, pose safety concerns, and have high energy consumption and low yields (Osman et al. 2024).

Nowadays researchers are exploring alternative methods to synthesize nanoparticles that are cost-effective, energy-efficient, and environmentally-friendly (Karim et al. 2023). The green synthesis method of nanoparticle synthesis is a novel approach that utilizes biological materials to be used as reducing, capping, and stabilizing agents in the synthesis reactions. The primary and secondary metabolites present in plants such as amino acids, proteins, vitamins and phenols, terpenoids, and flavonoids respectively are important reducing agents and capping to be used in the synthesis of nanoparticles (Silva et al. 2018). Plant extract of *Acalypha indica* (Krishnaraj et al. 2010), *Aloe vera* (Dinesh et al. 2015), *Nelumbo nucifera* (Santhoshkumar et al. 2011), and *Allium sativum* (Jini et al. 2022) have been reported to be used in the synthesis of silver nanoparticles. Leaf extract of *Melissa officianalis* has also been used as a reducing and capping agent in silver nanoparticle synthesis (de Jesús Ruíz-Baltazar et al. 2017). Synthesis of silver nanoparticles using seed extract of *Tectona grandis* was demonstrated by Rautela et al. (2019). Green synthesized nanoparticles have a wide range of applications in solving complex problems in the fields of medicine, pharmaceuticals, drug delivery, therapeutics, and environment degradation (Cuong et al. 2022). Gold nanoparticles synthesized using leaf extract of *Mentha longifera* demonstrated anti-human breast carcinoma properties (Li et al. 2021). Silver nanoparticles synthesized by *Acalypha indica* leaf extract have been tested against water-borne pathogens (Krishnaraj et al. 2010). Green synthesized Zinc and Copper nano-fertilizers have been demonstrated to have applications in agriculture and environmental sustainability (Abbasifar et al. 2020). Green synthesized gold and iron nanoparticles using *Syzigium cumini*, *Rosa indica*, and *Ficus macrocarpa* have been tested for targeted drug delivery potential (Khanzada et al. 2021).

Trachyspermum ammi commonly known as Ajwain (Apiacae family) is well known for its pharmacological and therapeutic uses (Saleem et al. 2017). It is a widely used antibacterial, antifungal, antifilarial, antipyretic, antioxidant, and anti-inflammatory herb (Kaur and Arora 2010). Thymol, myrcene, cervacrol, limonene, cymene, and terpinene are some bioactive compounds present in the herb, which act as reducing and capping agents in nanoparticle synthesis (Biswal et al. 2019). Synthesis of Magnesium oxide (MgO) nanoparticles using leaf extract of *Trachyspermum ammi* has been reported as an easy and eco-friendly nanoparticle synthesis approach (Dabhane et al. 2022). It has been suggested that *Trachyspermum ammi* leaf extract was used to create cobalt nanoparticles as well (Din et al. 2024).

The majority of eukaryotic cells are micrometers in size, whereas the size of the nanoparticles is in the region of nanometers. When nanoparticles get inside cells, they can disrupt normal biological functions (Mohammadinejad et al. 2019; Rasel et al. 2019). The potency of silver nanoparticles against larval stages of crop pests *Spodoptera frugiperda* was tested and significant mortality data was reported (Pittarate et al. 2023). Silver nanoparticles synthesized using *Lawsonia inermis* leaf extract have been shown to be effective against human head louse and sheep body louse with LC_{50} values of 1.33 and 1.41 ppm respectively (Marimuthu et al. 2012). Green silver nanoparticles showed adulticidal activity against *Haemaphysalis bispinosa* and *Hippobosca maculate* with LC_{50} values 2.30 ppm and 2.55 ppm respectively and LC 90 values 8.28 and 9.03 ppm respectively (Zahir and Rahuman 2012). Nanoparticles can be used against some disease-causing vector species that cause epidemics by spreading the disease in human habitations resulting in life-threatening medical and health situations. Outbreaks of such menace-causing vectors can be controlled using green synthesized nanoparticles against them to control their population around human habitations (Zargham et al. 2023).

Mosquito is one such disease-causing vector that brings havoc to human society by spreading life-threatening diseases like malaria, dengue, chikungunya, yellow fever, West Nile Fever, Filariasis, Japanese Encephalitis (Dahmana and Mediannikov 2020). The world has faced around 608000 deaths with a mortality rate of 14.3 deaths per 100000 people at risk in the year 2022 according to Venkatesan (2024). Since the inception of 2024, around 7.5 million cases and 3000 deaths were reported from 73 countries as per the European Centre for Disease Prevention and Control (2022). The Americas reported 3,684,554 cases of chikungunya in 50 countries in the 2013 to 2023 decade (de Souza et al. 2024). Worldwide, lymphatic filariasis poses a risk to 63% of 1.34 billion persons, among them 50% of infected persons belong to Southeast Asia (Bizhani et al. 2021). Annually, around 67,000 cases of Japanese Encephalitis are reported out of which 30% of the cases are fatal (Campbell et al. 2011). Exploring the potential of nanoparticles to be used as control tactics against mosquitoes can provide an alternative to traditional vector control strategies which utilize harmful chemicals like pyrethroids and organophosphates which pose risks to human health and the environment (Kaur et al. 2024).

This study is an attempt to provide an alternative mosquito vector control strategy using nanobiotechnology which involves a green synthesis of environmentally-friendly nanoparticles using leaf extract of the Ajwain plant, *Tachyspermum ammi* (TA), and testing their efficacy against larval and adult stages of three different genera; *Anopheles*, *Aedes* and *Culex* mosquitoes.

Methods

Collection of material

Fresh leaves of *Trachyspermum ammi* were collected from the Nursery, University of Rajasthan, Jaipur, and were taxonomically identified at the Herbarium, Department of Botany, University of Rajasthan, Jaipur. The herbarium sheet of the specimen was deposited in the Botany Department and voucher number RUBL21647 was received. The

leaves of the plant were washed properly in the laboratory successively with tap water followed by distilled water. After a thorough washing, the leaves were allowed to air dry at room temperature and ground into powdered form.

Preparation of plant extract

The aqueous extract was prepared by mixing 50 g of TA leaf powder with 200 ml of distilled water on a magnetic stirrer at 500 rpm at 40 °C for about three hours. The obtained mixture was allowed to cool at room temperature and a concentration was observed to be 250 mg/ml. For future use, the mixture was carefully filtered by Whatman filter paper No.1 and refrigerated at 4 °C.

Synthesis of TA-AgNPs

Analytical-grade silver nitrate was purchased from Sigma Aldrich. Aqueous 1 mM silver nitrate solution was prepared by mixing 17 mg of silver nitrate powder with 100 ml of distilled water and was stirred with a magnetic stirrer. TA-AgNPs were synthesized by taking 90 ml of 1 mM silver nitrate solution in a conical flask which was kept on a magnetic stirrer (Eltek Digimag). Under continuous stirring 10 ml of TA leaf extract was added drop by drop to the 90 ml of silver nitrate solution. The mixture was then left in dark conditions on a magnetic stirrer at 800 rpm and 35 °C temperature for 3 hours (Haris and Ahmad 2024).

Characterization

The size, shape, crystal structure, atomic composition, and associated functional groups in the synthesized nanoparticles were assessed by different characterization techniques. A sample from the synthesized solution was used to confirm the synthesis of nanoparticles through UV/Vis spectroscopy (Thermo Scientific Multiskan Go) under the 300 nm–600 nm range. Further, the mixture was centrifuged at 5,000 rpm for 15 minutes and the pellet was subsequently washed with distilled water and ethanol and was subjected to SEM analysis (Thermofisher Scientific Model Apreo 25 high vac). The EDX (Oxford EDX) spectra of the sample were also recorded. XRD (Panalytical Xpert Pro) was used to determine the crystalline structure of the synthesized nanoparticles. Functional groups associated with the nanoparticles were determined by FTIR using the KBr pellet method (Perkin Elmer 95163).

Test insects

Mosquito larvae of different genera; *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were reared in the laboratory using the standard protocol. Different larval genera were maintained in different enamel trays (40 × 30 × 8 cm) at 25 °C–28 °C. They were provided with dog biscuits and yeast extract as food in a ratio of 3:1. The mosquito adults were reared in rearing cages (1 × 1 × 1 m^3) in the laboratory. They were maintained at 25 °C–28 °C temperature and 75–80% relative humidity in the insectary. They were fed on 10% glucose solution soaked on cotton plugs. Blood feed was also provided 2 h a day by inserting hands in the rearing cages. Alternate light and dark conditions were maintained for a 14D:10L h period (Imam et al. 2014).

Larvicidal assay

With a little modification, the larvicidal assay was conducted following WHO (WHO 2005) standards for the laboratory testing of TA-AgNPs against mosquito larvae. Third and fourth instar mosquito larvae were subjected to quantities of green fabricated silver nanoparticles (TA-AgNPs-Group II) and leaf extract (TA-Group I) ranging from 0.5 ppm to 45 ppm. For the placebo group, distilled water was utilized (UN-Group III). Before preparing the dosages, the nanoparticles were ultrasonically dispersed to guarantee equal dispersion. In 250 ml of distilled water, the doses of Group I and Group II concentrations were established. In plastic cups, twenty-five mosquito larvae (third and fourth instars) of the following species: *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were exposed to different concentrations in Groups I, and II. For 24 hrs, mosquito larvae from each genus were treated in triplicate to the test concentration. After the exposure period ended, the immobile larvae were observed to record the mortality data of the treatment groups. The Abbott formula was applied to adjust the control group's mortality percentage that exceeded 5% as per WHO (2022).

$$\text{Corrected mortality} = \frac{\%\text{exposed mortality} - \%\text{control mortality}}{(100 - \%\text{control mortality})} \times 100$$

Adulticidal assay

Green synthesized silver nanoparticles (TA-AgNPs-Group II) and Trachyspermum ammi leaf extract (TA-Group I) at concentrations of 5 ppm, 20 ppm, 35 ppm, 75 ppm, and 95 ppm were applied to newly emerged adult stages of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* collected (F1 generation from rearing stock). In the control group, mosquitoes were exposed to distilled water (UN-Group III). To acclimate the mosquitoes to tubes, they were held in the holding tubes for one hour. Whatman filter papers (Analytical grade A) were treated with different test doses of leaf extract and silver nanoparticles for adulticidal experiments. The tests were conducted using WHO tube assay per the World Health Organization (2022). Filter paper in the control groups held only distilled water. Test tubes with treated filter papers have been

packed with 25 adult mosquitoes. For one hour, mosquitoes were placed in exposure tubes featuring treated filter paper and then in post-exposure holding tubes for 24 h. After the exposure period of 24 h, mortality data was seen by merely observing the knockdown adult mosquitoes that were resting on the test tube floor (Veerakumar et al. 2014).

Statistical analysis

The mean difference between the experimental groups was examined using MS Excel's One-way Analysis of Variance (ANOVA) function, and the average mean of the mortality data for larvae and adults was subjected to probit analysis to determine LC_{50}, LC_{90}, and LC_{95} values with 95% confidence limits. Plots of $p < 0.05$ and $p < 0.01$ indicated significant and extremely significant results, respectively.

Results

Characterization of TA-AgNPs

UV/Visible Spectroscopy

The color of the silver nitrate solution was changed from milky white to light green with the addition of plant extract and light green to black when the mixture was stirred for three hours. Synthesis of silver nanoparticles was confirmed by measuring the absorbance of the prepared solution. The absorbance peak was measured at 435 nm as depicted in Fig. 1.

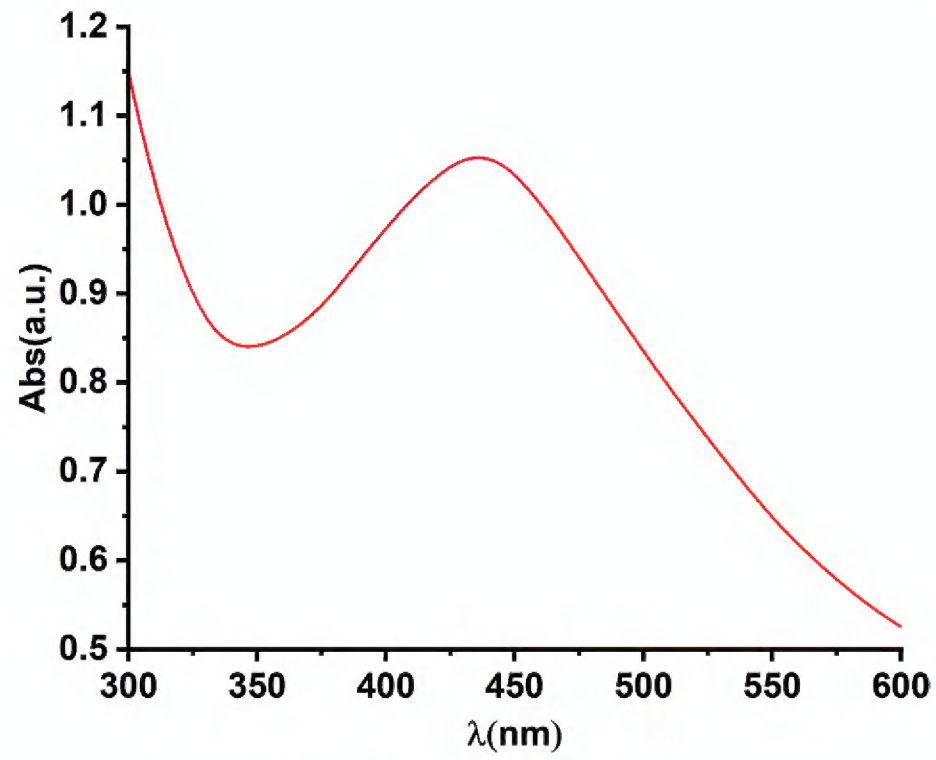


Figure 1. UV/Visible Spectrum of TA-AgNPs.

Field emission scanning electron microscope (FESEM)

The size and shape of the synthesized nanoparticles were determined using FESEM. The FESEM image (Fig. 2) depicted that the nanoparticles were spherical. The size of the nanoparticles was found to be in the range of 15–45 nm. The mean average size of the nanoparticles was found to be 27.9 nm (Fig. 2B).

Energy dispersive X-ray spectroscopy (EDX)

The EDX spectrum (Fig. 3) showed the presence of Ag (Silver), O (Oxygen), and N (Nitrogen). A strong peak at 3 keV reflects the presence of Silver in the sample. The weight %

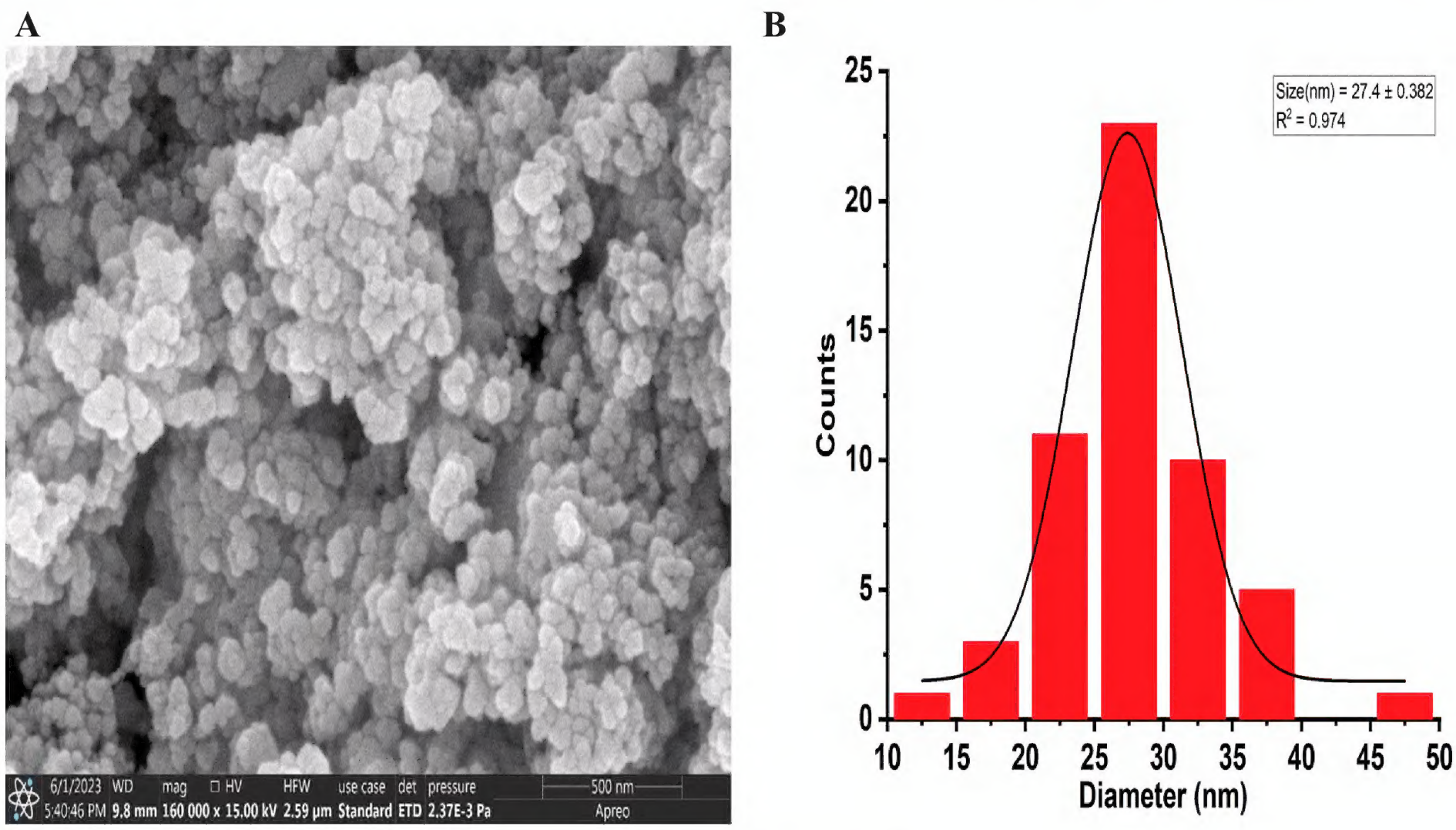
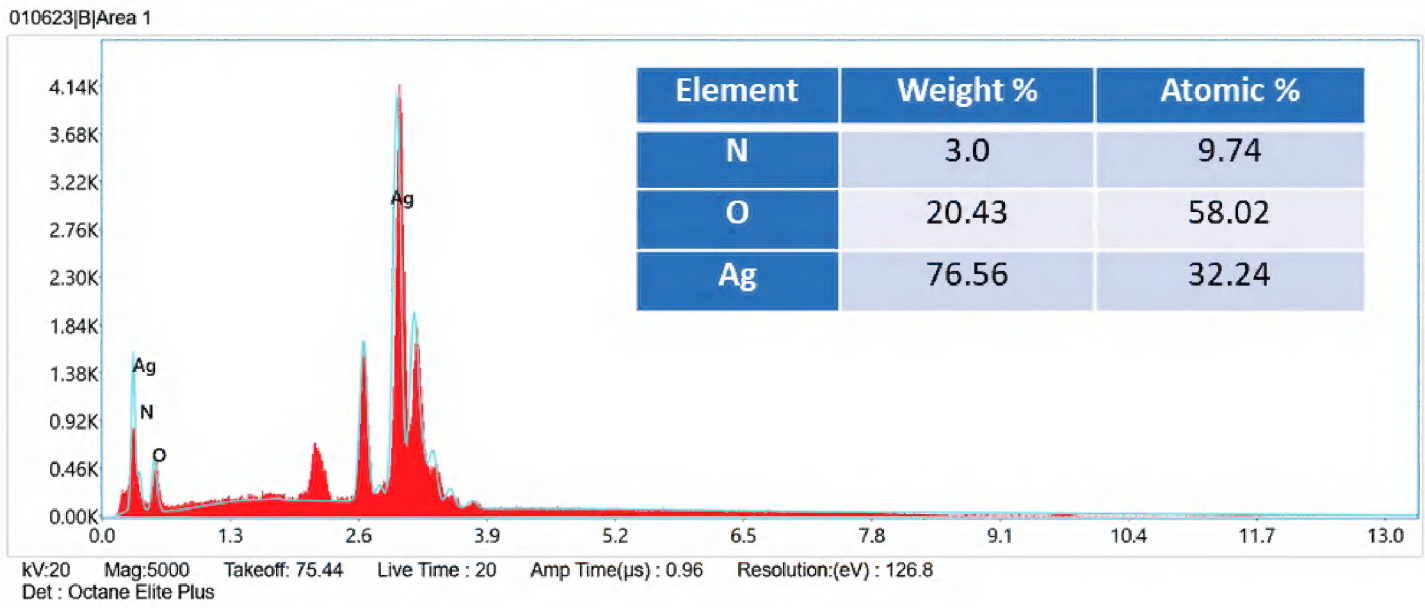


Figure 2. FESEM results of TA-AgNPs: **A.** SEM imaging; **B.** Mean average size.



Element	Weight %	Atomic %
N	3.0	9.74
O	20.43	58.02
Ag	76.56	32.24

Figure 3. EDX Spectra of TA-AgNPs.

and atomic % of Ag, O, and N were found to be 76.56%, 20.43%, 3.0%, and 32.24%, 58.02%, 9.74% respectively. A strong signal in the silver-containing region implies the synthesis of a major proportion of Silver nanoparticles.

X-ray diffraction pattern

The XRD data demonstrated different diffraction peaks corresponding to the 2θ values of 38.2°, 44.3°, 76.8° which can be assigned to planes of (1 1 1), (2 0 0), and (3 1 1) respectively (Fig. 4). The data shows that the synthesized TA-AgNPs are crystalline in nature (JCPDS file number-04-0783). A few unassigned peaks were also recorded that indicate the presence of crystal structures of the organic compounds on the surface of nanoparticles present in the leaf extract used for the synthesis process.

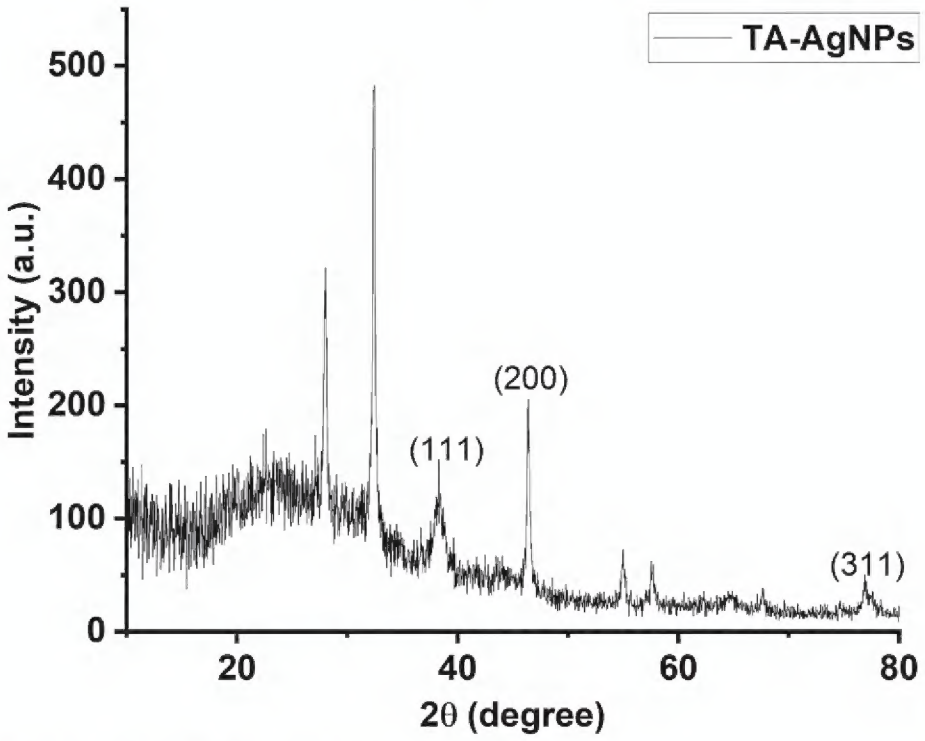


Figure 4. XRD Spectrum of TA-AgNPs.

Fourier transformation infrared spectroscopy

Analysis of FTIR data (Fig. 5) showed different peaks that corresponded to different functional groups attributed to the Silver Nanoparticles by the leaf extract. A strong peak at 3122.96 cm^{-1} showed the presence of O-H stretching, which corresponds to the presence of carboxyl or alcoholic group. Peak 2342.50 cm^{-1} corresponds to O=C=O stretching. Peaks at 615 cm^{-1} and 695 cm^{-1} showed C-Br stretching which signifies the presence of alkyl halide. A peak at 1448 cm^{-1} corresponded to N-H stretch vibrations and showed the presence of amide linkages present in proteins.

Larvicidal activity

The larvicidal activity of TA leaf extract and TA-AgNPs against third and fourth instar larval stages of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* mosquitoes, respectively, were conjectured in Table 1. When comparing the larvae treated with green synthesized nanoparticles (Group-II) to the crude leaf extract (Group-I) at all concentrations, a very high mortality rate was noted. Group III functioned as the control group, and no mortality was noted. *Anopheles* and *Aedes* showed comparable results at the dose of 0.5 ppm in Group I, with 2.67% mortality, while *Culex* had a marginally lower mortality rate (1.33%) among different genera. At the dose of 45 ppm for crude extract treatment *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* were observed with 30.66%, 34.67%, and 33.33% larval mortalities. Group II depicted a higher mortality percentage in all the genera *An. stephensi* (18.67%), *Ae. aegypti* (13.33%), and *Cx. quinquefasciatus* (12.00%) than Group I at the dose of 0.5 ppm. At the 45 ppm dose in *Anopheles*, all third and fourth larval instars were spotted to be dead. Compared to *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* had slightly lower larval mortality rates at the maximum dose—98.67% and 89.33%, respectively. Zero mortality was recorded in the control group therefore; Abbott's corrected mortality wasn't obtained.

Adulticidal activity

Table 2 indicated TA and TA-AgNPs' adulticidal activity against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* mosquito adult stages produced noteworthy outcomes. When exposed to TA-AgNPs (Group II) as opposed to Group-I, adult mosquitoes exhibited noticeably higher

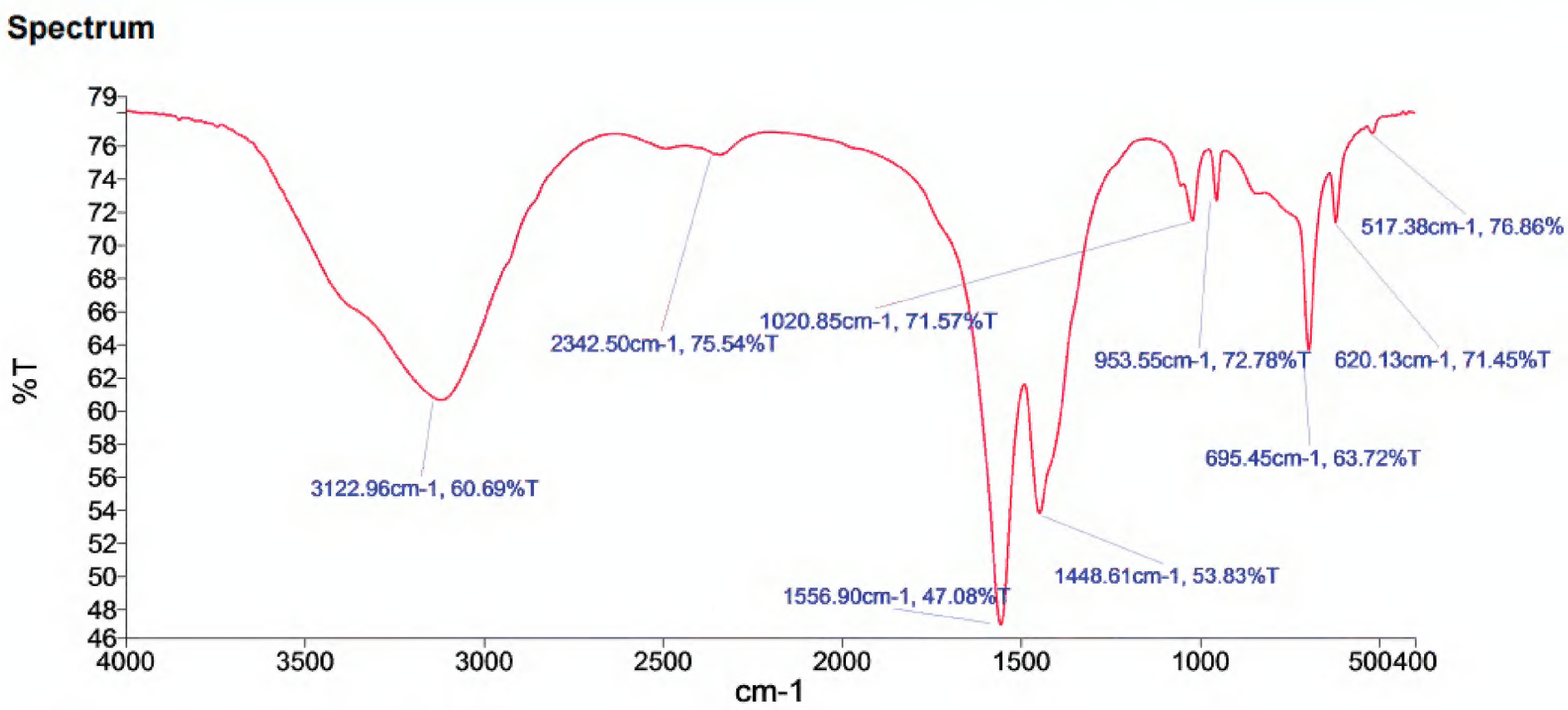


Figure 5. FTIR Spectrum of TA-AgNPs.

Table 1. Effect of plant extract and TA-AgNPs on larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*.

G	C	*An. stephensi*		*Ae. Aegypti*		*Cx. quinquefasciatus*	
		MA	% Mortality	MA	% Mortality	MA	% Mortality
G-I	0.5	0.67±0.33	2.67	0.67±0.33	2.67	0.33±0.33	1.33
	5	1.00±0.00	4.00	1.33±0.33	5.33	1.00±0.00	4.00
	15	1.67±0.33	6.67	2.33±0.33	9.33	2.33±0.33	9.33
	25	3.00±0.58	12.00	4.33±0.33	17.33	4.00±0.00	16.00
	35	5.33±0.33	21.33	7.33±0.33	29.33	7.00±0.00	28.00
	45	7.67±0.33	30.67	8.67±0.33	34.67	8.33±0.33	33.33
G-II	0.5	4.67±0.33	18.67	3.33±0.33	13.33	3.00±0.00	12.00
	5	7.33±0.33	29.33	5.67±0.33	22.67	5.67±0.33	22.67
	15	9.33±0.33	37.33	8.67±0.33	34.67	8.33±0.33	33.33
	25	14.00±0.58	56.00	13.00±0.58	52.00	12.67±0.33	50.67
	35	20.00±0.58	80.00	21.67±0.33	86.67	18.00±0.58	72.00
	45	25.00±0.00	100.00	24.67±0.33	98.67	22.33±0.33	89.33
G-III	P	0.00	0.00	0.00	0.00	0.00	0.00

G-I: TA-Plant extract group, G-II: TA-AgNPs group, G-III (P): Placebo group, C – Concentration (in ppm), MA – Mean Average (Note – Three replicates with 25 larvae taken for each species each replicate).

Table 2. Effect of plant extract and TA-AgNPs on adults of *An. stephensi*, *Ae. aegypti*, and *Cx. Quinquefasciatus*.

G	C	*An. stephensi*		*Ae. Aegypti*		*Cx. quinquefasciatus*	
		MA	% Mortality	MA	% Mortality	MA	% Mortality
G-I	5	4.67±0.33	18.67	3.67±0.33	14.67	3.33±0.33	13.33
	20	7.67±0.33	30.67	7.33±0.33	29.33	6.67±0.58	26.67
	35	10.67±0.33	42.67	10.33±0.33	41.33	8.33±0.33	33.33
	55	12.67±0.58	50.67	12.33±0.33	49.33	10.33±0.58	41.33
	75	15.67±0.33	62.67	15.33±0.33	61.33	12.67±0.33	50.67
	95	18.67±0.33	74.67	18.33±0.33	73.33	14.33±0.33	57.33
G-II	5	7.67±0.33	30.67	6.00±0.67	24.00	5.67±0.33	22.67
	20	9.67±0.33	38.67	9.33±0.58	37.33	8.67±0.00	34.67
	35	12.67±0.33	50.67	12.33±0.33	49.33	11.67±0.33	46.67
	55	16.00±0.33	64.00	15.00±0.58	60.00	14.67±0.33	58.67
	75	20.00±0.33	80.00	19.00±0.33	76.00	18.67±0.33	74.67
	95	25.00±0.33	100.00.	22.67±0.33	90.67	22.33±0.33	89.33
G-III	P	0.00	000	0.00	0.00	0.00	0.00

G-I: TA-Plant extract group, G-II: TA-AgNPs group, G-III (P): Placebo group, C – Concentration (in ppm), R – Replicate, MA – Mean Average (Note – Three replicates with 25 adults taken for each species, each replicate).

adulticidal activity. There was no recorded mortality percentage in the control group. The adult mortality percentages of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* depicted results with 18.67%, 14.67%, 13.33% at the dose of 5 ppm and 74.67%, 73.33%, 57.33% at the dose of 95 ppm in treatment with crude plant extracts. Comparably, the data findings from the tables displayed that the mortality percentage of 30.67%, 24%, 22.67% and 100%, 90.67%, 89.33% at the lowest dose of 5 ppm and highest dose of 95 ppm in *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. *An. stephensi* (Group-II) had the highest mortality percentage (100%) when all the doses' mortality percentages were compared, indicating that the TA-AgNPs generated was an effective insecticide at suppressing the mosquito population. Zero mortality was recorded in the control group therefore; Abbott's corrected mortality wasn't obtained.

Statistical analysis

One-way ANOVA was implemented to compare the treated and untreated groups for larval and adult mortality. The results revealed values of $p<0.05$ in all genera, indicating highly significant findings. When tested against larvae and adults of different genera, the TA-AgNPs' effectiveness was, however, evaluated as being more highly susceptible than the crude extracts. One-way ANOVA followed by probit plane regression analysis was conducted to determine the LC_{50}, LC_{90}, and LC_{95} for Group I and Group II independently. Various dose concentrations at different endpoints of lethal concentration (LC_{50}, LC_{90}, and LC_{95}) depicted that the lowest dose required to produce acute toxicity for 50%, 90%, and 95% mortalities of larva mosquito populations in *An. stephensi* (LC_{50}- 33.27, LC_{90}-62.19, and LC_{95}-65.80) and *Ae. aegypti* (LC_{50}-33.26 LC_{90}-62.05, and LC_{95} -65.66) are almost similar as compared to *Cx. quinquefasciatus* (LC_{50}- 43.20447, LC_{90}-80.61 and LC_{95} -85.29) in Group-II data results. *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* had LC_{50}, LC_{90}, and LC_{95} values in Group I of the larvicidal assay of 74.70 ppm, 138.40 ppm, and 146.36 ppm; 64.28 ppm, 119.13 ppm, and 125.99 ppm; 50.11 ppm, 92.56 ppm, and 97.87 ppm, respectively. The fact that Group II had LC_{50}, LC_{90}, and LC_{95} values lower than Group confirmed that TA-AgNPs have greater larvicidal activity than TA-extract. The two genera with the lowest documented lethal concentrations (G-II) were *Anopheles* spp. and *Aedes* spp. followed by *Culex* spp.

The adult mortality estimates for Group II showed an analogous trend for both LC_{90} and LC_{95}. As anticipated and noted, the lethal concentration values for all three genera were higher in Group II than in Group I. The LC_{50}, LC_{90}, and LC_{95} estimates for TA-AgNPs for adulticidal assay in *An. stephensi* was calculated to be 41.14 ppm, 72.28 ppm, 80.67 ppm, and 23.32 ppm, 42.96 ppm, 45.42 ppm in Group I and Group II, respectively. These results were lower than those for *Ae. Aegypti* (LC_{50}= 38.72, LC_{90}=76.67, LC_{95}=75.79 ppm for Group I, and LC_{50}= 32.43, LC_{90}=60.02, LC_{95}=63.47 ppm for Group II), and *Cx. quinquefasciatus* (LC_{50}= 47.19, LC_{90}=87.45, LC_{95}=92.49 ppm for Group I assay, and LC_{50}= 33.18, LC_{90}=61.42, LC_{95}= 64.95 ppm for Group II assay), indicating that TA-AgNPs are more effective against *An. stephensi* mosquitoes than *Ae. aegypti* and *Cx. quinquefasciatus*. Table 3 displays the statistical summary for the larval and adult stages for both groups (G-I, G-II).

Figs 6–11 showed the probability output in the form of graphs with sample percentile and probit analysis, where each group's larvicidal and adulticidal efficiency is compared with the control. The histograms compare the adult and larval mortality percentage in both treated groups with a placebo and the degree of significance (Figs 12, 13).

Table 3. Statistical interpretations of both the treated groups (G-I, G-II) for larvae and adults against various genera.

G	*An. stephensi*	*Ae. Aegypti*	*Cx. quinquefasciatus*
G-I (L)	Y = 0.627982038x + 3.087636777	Y = 0.72922x + 3.12521	Y = 0.942136x + 2.792954
G-II (L)	Y = 1.38336x + 3.96428	Y = 1.389391x + 3.776416	Y = 1.069322x + 3.800514
SE (L)	0.315464	0.296726	0.228071
SE (L)	1.163613	0.899615	0.48318
G-I (A)	Y = 1.138184x + 3.178362	Y = 1.213999x + 2.991238	Y = 1.993413x + 3.120496
G-II (A)	Y = 2.036979x + 2.487947	Y = 1.449733x + 2.990579	Y = 1.416389x + 3.003025
SE (A)	0.183703	0.161045	0.228071
SE (A)	1.003987	0.343689	0.48318

Where G – Group, L – Larva, A – adult, Y = ax + b shows the probit equation, SE – Standard error.

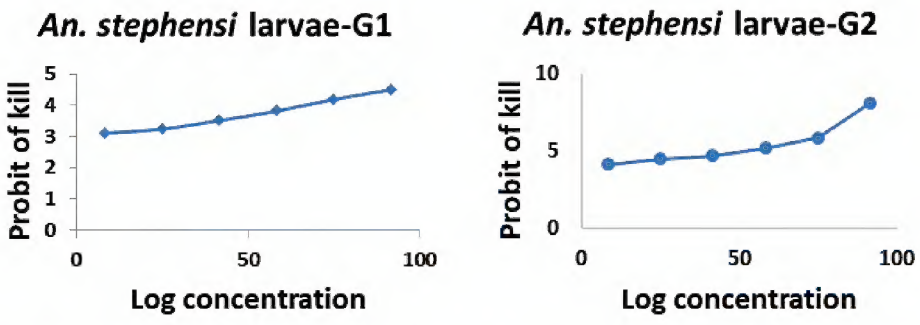


Figure 6. Probit plots for *An. stephensi* larvae in both the treated groups.

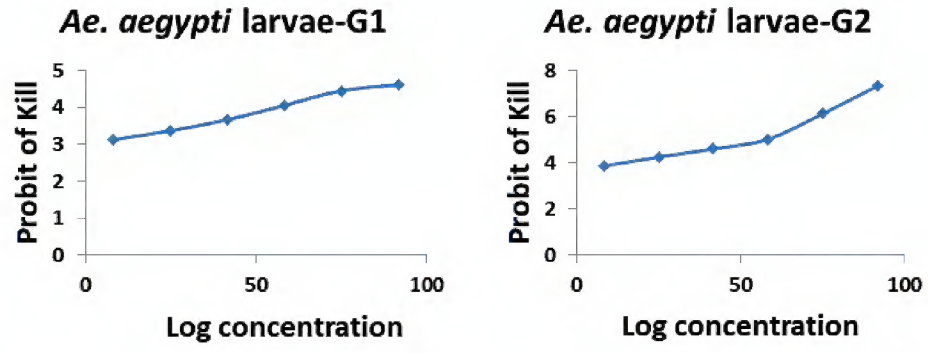


Figure 7. Probit plots for *Ae. aegypt* larvae in both the treated groups.

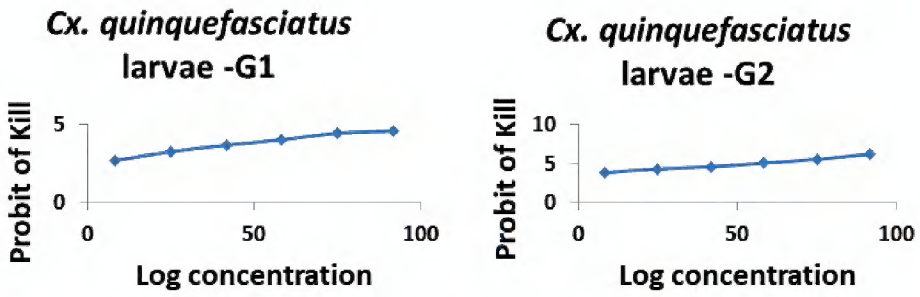


Figure 8. Probit plots for *Cx. quinquefasciatus* larvae in both the treated groups.

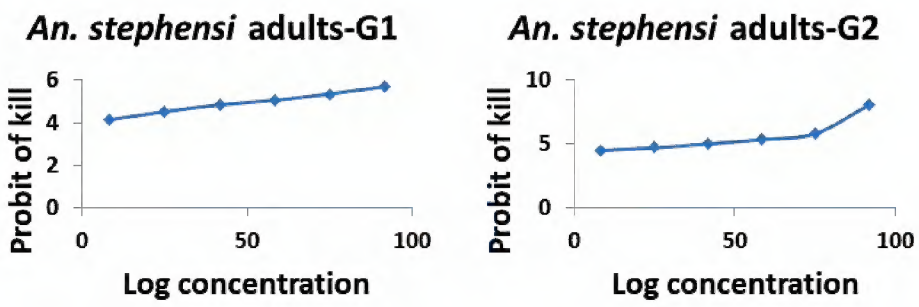


Figure 9. Probit plots for *An. stephensi* adults in both the treated groups.

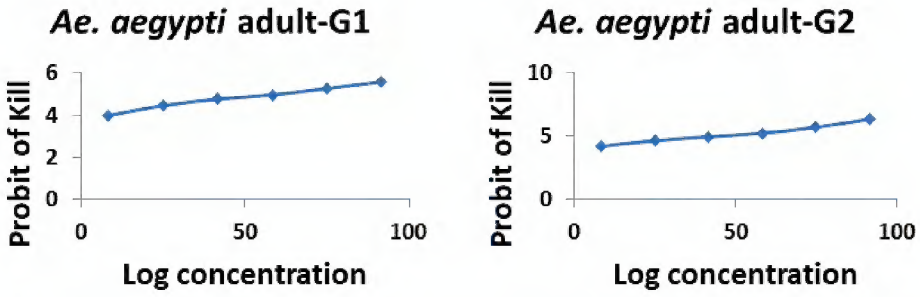


Figure 10. Probit plots for *Ae. aegypti* adults in both the treated groups.

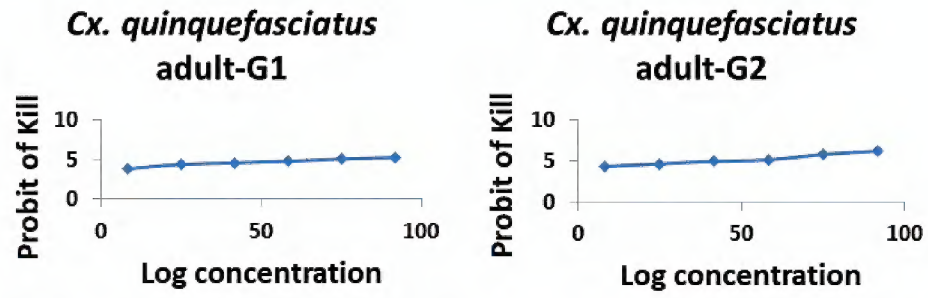


Figure 11. Probit plots for *Cx. quinquefasciatus* adults in both the treated groups.

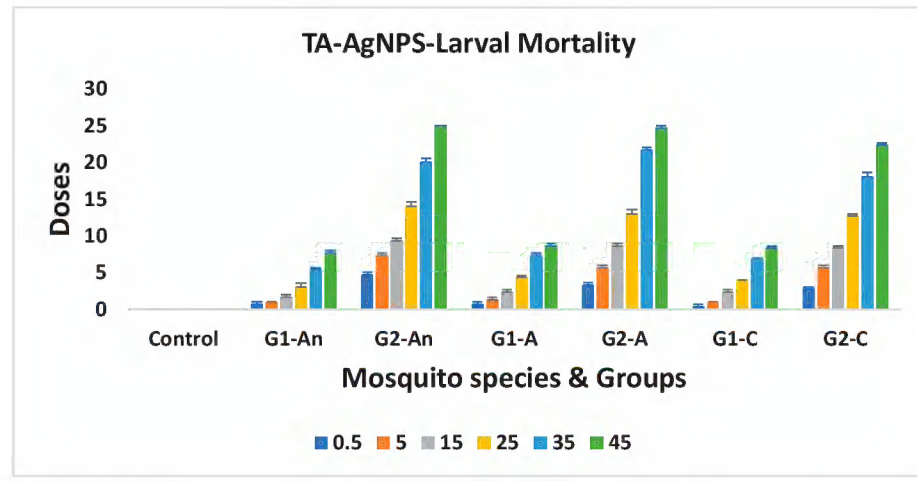


Figure 12. A Histogram to show the bioassay results for treated larvae of different genera (where G1 and G2 are the groups, *An – Anopheles*, *A – Aedes*, *C – Culex*).

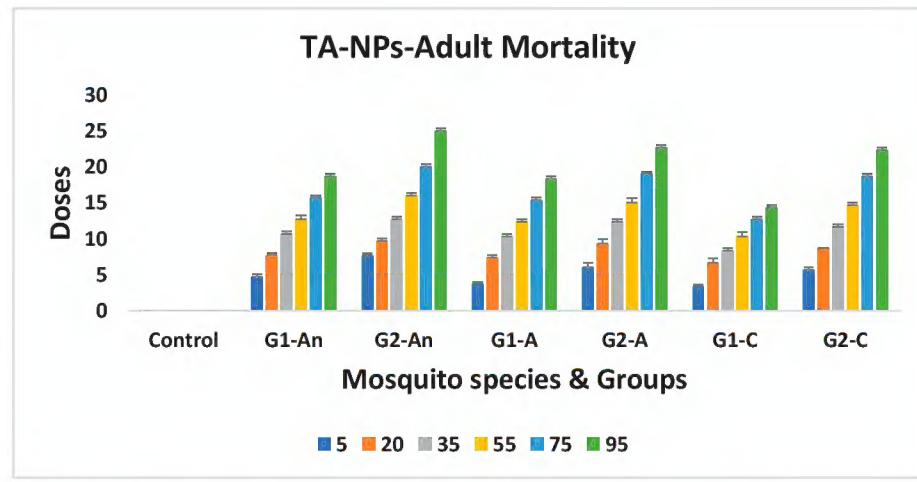


Figure 13. A Histogram to show the bioassay results for treated adults of different genera (where G1 and G2 are the groups, *An – Anopheles*, *A – Aedes*, *C – Culex*).

Discussion

Recently, nanoparticle synthesis methods have shifted to a green and environmentally-friendly approach using parts of plants as reducing, capping, and stabilizing agents (Selvaraj et al. 2021). Many methods are employed to characterize the produced nanoparticles. There have been reports of the manufacture of silver nanoparticles from other plants. Leaf extract of *Acalypha indica* has also been reported for the synthesis and characterization of silver nanoparticles and their antimicrobial activity against water-borne pathogens. According to a study (Krishnaraj et al. 2010), the absorbance peak of 420 nm was seen by the silver nanoparticles in the UV/visible spectrophotometer; nevertheless, our results indicated an absorbance peak of 435 nm. A similar absorbance peak was observed in silver nanoparticles synthesized using *Prosopis julifora* leaf extract (Raja et al. 2012). Using an aqueous extract of *Chlorella vulgaris* and fenugreek leaf extract, silver nanoparticles were generated (Arsiya et al. 2017; Ghoshal and Singh 2022). Their size and shape were examined using a FESEM, and the results were consistent with our findings.

When AgNPs made with *Morinda lucida* leaf extract were elementally examined using EDX, it revealed a significant peak for silver and a few weak peaks for elements like O, N, C, P, and Cl (Labulo et al. 2022). Most likely, *Cucumis phrophetarum* leaf extract revealed the presence of oxygen, carbon, and silver. According to a study (Hemlata et al. 2020) estimation, a significant peak at 3 eV indicated the presence of silver in EDX spectra. These results correspond to the EDX spectra of the present study which demarcated the presence of Nitrogen and Oxygen, along with the Silver, and reflected the contribution of organic compounds in the leaf extract of TA to the silver nanoparticles. Microorganisms like *Psuedomonas fluorescens* can be deployed to make AgNPs, not just plants (Kalaimurugan et al. 2019). According to Ramaswamy and his co-workers (Ramaswamy et al. 2015), the FTIR spectra of silver nanoparticles generated using *Vernonia cineraria* showed peaks at 2129 cm^{-1}, 1737 cm^{-1}, 1440 cm^{-1}, 1365 cm^{-1}, and 1218 cm^{-1} that were associated with silver nanoparticles. Another study revealed AgNPs using *Myrsine africana* leaf extract showed FTIR bands at 1699 cm^{-1}, 1558 cm^{-1}, 1479 cm^{-1}, 1392 cm^{-1}, 1047 cm^{-1}, 646 cm^{-1}, 534 cm^{-1} which illustrated to C=C, C=O, C-C, -OH, C-N, C-X stretching respectively (Sarwer et al. 2022). Bands of these functional groups depicted the presence of biomolecules acting as capping as well as stabilizing agents that reduce Ag+ to

Ag nanoparticles (Loo et al. 2018). When biomolecules of TA leaf extract were added to the nanoparticles, TA-Ag-NPs revealed FTIR bands that verified the presence of alkanes, carboxyl groups, alkanes, hydroxyl groups, amide groups, and alkyl halides.

Leaf extract of *Tridax procumbens* has shown LC_{50} and LC_{90} values as 51.57 mg/l and 226.56 ppm respectively against *Anopheles* larvae and 42.29 and 172.34 ppm respectively against *Culex* larvae (Kamaraj et al. 2011). Silver nanoparticles synthesized using *Jasminum nervosum* showed LC_{50} -57.40 ppm against *Culex* larvae (Lallawmawma et al. 2015). *Trachyspermum ammi* crude leaf extract produced subpar results in a larvicidal assay, however, TA-AgNPs performed better and exhibited greater efficiency in the present investigation.

Extract of *Azadirachta indica* showed LC_{50} value of 106.65 ppm against adult stages of *Anopheles* mosquito. When adult stages of *Culex* mosquito were exposed to *Achyranthus aspera* and *Convulvulus arvensis* LC_{50} values were 87 and 300 ppm respectively (Zulhussnain et al. 2020). Silver nanoparticles synthesized using *Phyllanthus niruri* plant extract showed adulticidal activity against *Aedes* mosquito with LC_{50} and LC_{90} values of 6.68 and 23.58 ppm respectively (Suresh et al. 2015). LC_{50} values for adult stages of *Anopheles* and *Aedes* mosquito, when exposed to silver nanoparticles synthesized using *Mimusops elengi* were found to be 13.7 and 14.7 ppm respectively (Subramaniam et al. 2015). Comparable outcomes were observed in the targeted investigation, wherein TA-AgNPs demonstrated a high susceptibility to *An. stephensi* and *Ae. aegypti*, but a modest susceptibility to *Cx. quinquefasciatus*. The Nanoparticles are known to cause the histological and morphological abnormalities in the cuticle and result in dehydration and deformation in larvae of *Anopheles stutzeri* and *Culex quinquefasciatus* (Nie et al. 2023). The *Ae. aegypti* larval stages when exposed to the ZnO nanoparticles caused midgut lesions, abdominal contractions, and loss of anal gills or hairs due to the agglomeration in the abdomen (Banumathi et al. 2017). The study lacks evidence of the mode of action of TA-AgNPs in insect bodies. To determine which body portion of the test insect TA-AgNPs are tagged to, more research activities are under pipeline to be carried out.

Conclusion

The study showed that the green synthesis method—which uses *Trachyspermum ammi* leaf extract—is a simple, economical, energy-efficient, and environmentally friendly way to produce silver nanoparticles. Moreover, the ability of silver nanoparticles to be used in vector control programs as a cutting-edge method of controlling mosquito populations has demonstrated the effectiveness of these particles against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* mosquito larval and adult stages. Governments will be less dependent on bioaccumulating and biomagnifying pesticides to control mosquito populations and disease outbreaks in human habitations as a result of our assistance in developing alternative mosquito control programs and policies. Future studies have a significant scope in exploring the mechanism of action of silver nanoparticles in mosquito bodies. Additional research on how nanoparticles affect non-target creatures will shed additional light on the practical use of nanoparticles in vector population control initiatives.

Acknowledgments

The authors are grateful to the University of Rajasthan nursery workers for their assistance in gathering the source plant material. We also acknowledge the Department of Botany, University of Rajasthan, Jaipur for their thoughtful assistance in the taxonomic identification of the collected source plant material.

Grant Information: Authors have no funding to report.

References

Abbasifar A, Shahrabadi F, ValizadehKaji B (2020) Effects of green synthesized zinc and copper nano-fertilizers on the morphological and biochemical attributes of basil plant. Journal of Plant Nutrition 43(8): 1104–1118. https://doi.org/10.1080/01904167.2020.1724305

Arsiya F, Sayadi MH, Sobhani S (2017) Green synthesis of palladium nanoparticles using *Chlorella vulgaris*. Materials Letters 186: 113–115. https://doi.org/10.1016/j.matlet.2016.09.101

Banumathi B, Vaseeharan B, Ishwarya R, Govindarajan M, Alharbi NS, Kadaikunnan S, Khaled JM, Benelli G (2017) Toxicity of herbal extracts used in ethno-veterinary medicine and green-encapsulated ZnO nanoparticles against *Aedes aegypti* and microbial pathogens. Parasitology Research 116: 1537–1551. https://doi.org/10.1007/s00436-017-5438-6

Biswal RA, Venkataraghavan R, Pazhamalai V, Romauld SI (2019) Molecular docking of various bioactive compounds from essential oil of *Trachyspermum ammi* against the fungal enzyme Candidapepsin-1. Journal of Applied Pharmaceutical Science 9(5): 21–32. https://doi.org/10.7324/JAPS.2019.90503

Bizhani N, Hashemi Hafshejani S, Mohammadi N, Rezaei M, Rokni MB (2021) Lymphatic filariasis in Asia: a systematic review and meta-analysis. Parasitology Research 120: 411–422. https://doi.org/10.1007/s00436-020-06991-y

Campbell GL, Hills SL, Fischer M, Jacobson JA, Hoke CH, Hombach JM, Marfin AA, Solomon T, Tsai TF, Tsu VD, Ginsburg AS (2011) Estimated global incidence of Japanese encephalitis: A systematic review. Bulletin of the World Health Organization 89(10): 766–774. https://doi.org/10.2471/BLT.10.085233

Colson P, Henrist C, Cloots R (2013) Nanosphere lithography: A powerful method for the controlled manufacturing of nanomaterials. Journal of Nanomaterials 948510: 1–19. https://doi.org/10.1155/2013/948510

Cong C, Nakayama S, Maenosono S, Harada M (2018) Microwave-assisted polyol synthesis of Pt/Pd and Pt/Rh bimetallic nanoparticles in polymer solutions prepared by batch and continuous-flow processing. Industrial and Engineering Chemistry Research 57(1): 179–190. https://doi.org/10.1021/acs.iecr.7b03154

Cuong HN, Pansambal S, Ghotekar S, Oza R, Thanh Hai NT, Viet NM, Nguyen VH (2022) New frontiers in the plant extract mediated biosynthesis of copper oxide (CuO) nanoparticles and their potential applications: A review. Environmental Research 203: 111858. https://doi.org/10.1016/j.envres.2021.111858

Dabhane H, Ghotekar S, Zate M, Kute S, Jadhav G, Medhane V (2022) Green synthesis of MgO nanoparticles using aqueous leaf extract of Ajwain (*Trachyspermum ammi*) and evaluation of their catalytic and biological activities. Inorganic Chemistry Communications 138: 109270. https://doi.org/10.1016/j.inoche.2022.109270

Dahmana H, Mediannikov O (2020) Mosquito-borne diseases emergence/resurgence and how to effectively control it biologically. Pathogens 9(4): 310. https://doi.org/10.3390/pathogens9040310

Din MI, Hussain Z, Siddique N, Sharif A, Intisar A, Ahmed E, Arshad M (2024) Sustainable synthesis of cobalt nanoparticles in the presence of *Trachyspermum ammi* leaf extract for water purification. Desalination and Water Treatment 317: 100100. https://doi.org/10.1016/j.dwt.2024.100100

Dinesh D, Murugan K, Madhiyazhagan P, Panneerselvam C, Mahesh Kumar P, Nicoletti M, Jiang W, Benelli G, Chandramohan B, Suresh U (2015) Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from *Aloe vera* extracts: towards an effective tool against the malaria vector *Anopheles stephensi*? Parasitology Research 114: 1519–1529. https://doi.org/10.1007/s00436-015-4336-z

European Centre for Disease Prevention and Control (2022) Dengue worldwide overview. Surveillance and disease data.

Ghoshal G, Singh M (2022) Characterization of silver nano-particles synthesized using fenugreek leave extract and its antibacterial activity. Materials Science for Energy Technologies 5: 22–29. https://doi.org/10.1016/j.mset.2021.10.001

Haris Z, Ahmad I (2024) Green synthesis of silver nanoparticles using *Moringa oleifera* and its efficacy against gram-negative bacteria targeting quorum sensing and biofilms. Journal of Umm Al-Qura University for Applied Sciences 10: 156–167. https://doi.org/10.1007/s43994-023-00089-8

Hemlata, Meena PR, Singh AP, Tejavath KK (2020) Biosynthesis of silver nanoparticles using *Cucumis prophetarum* aqueous leaf extract and their antibacterial and antiproliferative activity against cancer cell lines. ACS Omega 5(10): 5520–5528. https://doi.org/10.1021/acsomega.0c00155

Imam H, Sofi G, Zarnigar, Aziz S (2014) The basic rules and methods of mosquito rearing (*Aedes aegypti*). Tropical Parasitology 4(1): 53–55. https://doi.org/10.4103/2229-5070.129167

de Jesús Ruíz-Baltazar Á, Reyes-López SY, Larrañaga D, Estévez M, Pérez R (2017) Green synthesis of silver nanoparticles using a *Melissa officinalis* leaf extract with antibacterial properties. Results in Physics 7: 2639–2643. https://doi.org/10.1016/j.rinp.2017.07.044

Jiang P, Zhu DL, Zhu CN, Zhang ZL, Zhang GJ, Pang DW (2015) A highly reactive chalcogenide precursor for the synthesis of metal chalcogenide quantum dots. Nanoscale 7: 19310–19316. https://doi.org/10.1039/C5NR05747G

Jini D, Sharmila S, Anitha A, Pandian M, Rajapaksha RMH (2022) In vitro and in silico studies of silver nanoparticles (AgNPs) from *Allium sativum* against diabetes. Scientific Reports 12: 22109. https://doi.org/10.1038/s41598-022-24818-x

Johans C, Clohessy J, Fantini S, Kontturi K, Cunnane VJ (2002) Electrosynthesis of polyphenylpyrrole coated silver particles at a liquid-liquid interface. Electrochemistry Communications 4(3): 227–230. https://doi.org/10.1016/S1388-2481(02)00256-4

Kalaimurugan D, Vivekanandhan P, Sivasankar P, Durairaj K, Senthilkumar P, Shivakumar MS, Venkatesan S (2019) Larvicidal activity of silver nanoparticles synthesized by *Pseudomonas fluorescens* YPS3 isolated from the eastern ghats of India. Journal of Cluster Science 30: 225–233. https://doi.org/10.1007/s10876-018-1478-z

Kamaraj C, Bagavan A, Elango G, Zahir AA, Rajakumar G, Marimuthu S, Santhoshkumar T, Rahuman AA (2011) Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. Indian Journal of Medical Research 134(1): 101–106. https://pubmed.ncbi.nlm.nih.gov/21808141/

Karim N, Liu S, Rashwan AK, Xie J, Mo J, Osman AI, Rooney DW, Chen W (2023) Green synthesis of nanolipo-fibersomes using Nutriose® FB 06 for delphinidin-3-O-sambubioside delivery: Characterization, physicochemical properties, and application. International Journal of Biological Macromolecules 247:125839. https://doi.org/10.1016/j.ijbiomac.2023.125839

Kaur GJ, Arora DS (2010) Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbelliferae – Current status. Journal of Medicinal Plants Research 4(2): 87–94. http://www.academicjournals.org/JMPR

Kaur R, Choudhary D, Bali S, Bandral SS, Singh V, Ahmad MA, Rani N, Singh TG, Chandrasekaran B (2024) Pesticides: An alarming detrimental to health and environment. Science of the Total Environment 915: 170113. https://doi.org/10.1016/j.scitotenv.2024.170113

Khanzada B, Akthar N, Bhatti MZ, Ismail H, Alqarni M, Mirza B, Mostafa-Hedeab G, Batiha GES (2021) Green synthesis of gold and iron nanoparticles for targeted delivery: An *in vitro* and *in vivo* study. Journal of Chemistry 12021: 581444. https://doi.org/10.1155/2021/1581444

Khashan KS, Sulaiman GM, Abdulameer FA (2016) Synthesis and antibacterial activity of CuO nanoparticles suspension induced by laser ablation in liquid. Arabian Journal for Science and Engineering 41: 301–310. https://doi.org/10.1007/s13369-015-1733-7

Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N (2010) Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces 76(1): 50–56. https://doi.org/10.1016/j.colsurfb.2009.10.008

Labulo AH, David OA, Terna AD (2022) Green synthesis and characterization of silver nanoparticles using *Morinda lucida* leaf extract and evaluation of its antioxidant and antimicrobial activity. Chemical Papers 76: 7313–7325. https://doi.org/10.1007/s11696-022-02392-w

Lallawmawma H, Sathishkumar G, Sarathbabu S, Ghatak S, Sivaramakrishnan S, Gurusubramanian G, Kumar NS (2015) Synthesis of silver and gold nanoparticles using *Jasminum nervosum* leaf extract and its larvicidal activity against filarial and arboviral vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Environmental Science and Pollution Research 22: 17763–17768. https://doi.org/10.1007/s11356-015-5001-x

Li S, Al-Misned FA, El-Serehy HA, Yang L (2021) Green synthesis of gold nanoparticles using aqueous extract of *Mentha longifolia* leaf and investigation of its anti-human breast carcinoma properties in the in vitro condition. Arabian Journal of Chemistry 14(2): 102931. https://doi.org/10.1016/j.arabjc.2020.102931

Loo YY, Rukayadi Y, Nor-Khaizura MAR, Kuan CH, Chieng BW, Nishibuchi M, Radu S (2018) *In vitro* antimicrobial activity of green synthesized silver nanoparticles against selected Gram-negative foodborne pathogens. Frontiers in Microbiology 9: 1555. https://doi.org/10.3389/fmicb.2018.01555

Marimuthu S, Rahuman AA, Santhoshkumar T, Jayaseelan C, Kirthi AV, Bagavan A, Kamaraj C, Elango G, Zahir AA, Rajakumar G, Velayutham K (2012) Lousicidal activity of synthesized silver nanoparticles using *Lawsonia inermis* leaf aqueous extract against *Pediculus humanus capitis* and *Bovicola ovis*. Parasitology Research 111: 2023–2033. https://doi.org/10.1007/s00436-011-2667-y

Mascolo MC, Pei Y, Ring TA (2013) Room temperature co-precipitation synthesis of magnetite nanoparticles in a large pH window with different bases. Materials 6(12): 5549–5567. https://doi.org/10.3390/ma6125549

McGilvray KL, Decan MR, Wang D, Scaiano JC (2006) Facile photochemical synthesis of unprotected aqueous gold nanoparticles. Journal of the American Chemical Society 128(50): 15980–15981. https://doi.org/10.1021/ja066522h

Medina-Pérez G, Fernández-Luqueño F, Vazquez-Nuñez E, López-Valdez F, Prieto-Mendez J, Madariaga-Navarrete A, Miranda-Arámbula M (2019) Remediating polluted soils using nanotechnologies: Environmental benefits and risks. Polish Journal of Environmental Studies 28(3): 1013–1030. https://doi.org/10.15244/pjoes/87099

Mohammadinejad R, Moosavi MA, Tavakol S, Vardar DÖ, Hosseini A, Rahmati M, Dini L, Hussain S, Mandegary A, Klionsky DJ (2019) Necrotic, apoptotic and autophagic cell fates triggered by nanoparticles. Autophagy 15: 4–33. https://doi.org/10.1080/15548627.2018.1509171

Nie D, Li J, Xie Q, Ai L, Zhu C, Wu Y, Gui Q, Zhang L, Tan W (2023) Nanoparticles: A potential and effective method to control insect-borne diseases. Bioinorganic Chemistry and Applications 2023: 5898160. https://doi.org/10.1155/2023/5898160

Osman AI, Zhang Y, Farghali M, Rashwan AK, Eltaweil AS, Abd El-Monaem EM, Mohamed IMA, Badr MM, Ihara I, Rooney DW, Yap PS (2024) Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. Environmental Chemistry Letters 22: 841–887. https://doi.org/10.1007/s10311-023-01682-3

Pittarate S, Perumal V, Kannan S, Mekchay S, Thungrabeab M, Suttiprapan P, Sengottayan SN, Krutmuang P (2023) Insecticidal efficacy of nanoparticles against *Spodoptera frugiperda* (J.E. Smith) larvae and their impact in the soil. Heliyon 9(5): e16133. https://doi.org/10.1016/j.heliyon.2023.e16133

Raja K, Saravanakumar A, Vijayakumar R (2012) Efficient synthesis of silver nanoparticles from *Prosopis juliflora* leaf extract and its antimicrobial activity using sewage. Spectrochimica Acta – Part A: Molecular and Biomolecular Spectroscopy 97: 490–494. https://doi.org/10.1016/j.saa.2012.06.038

Ramaswamy U, Mukundan D, Sreekumar A, Mani V (2015) Green synthesis and characterization of silver nanoparticles using aqueous whole plant extract of *Vernonia cinerea* L. and its biological activities. In: Materials Today: Proceedings 2(9): 4600–4608. https://doi.org/10.1016/j.matpr.2015.10.080

Rasel MAI, Singh S, Nguyen TD, Afara IO, Gu Y (2019) Impact of nanoparticle uptake on the biophysical properties of cell for biomedical engineering applications. Scientific Reports 9: 5859. https://doi.org/10.1038/s41598-019-42225-7

Rautela A, Rani J, Debnath (Das) M (2019) Green synthesis of silver nanoparticles from *Tectona grandis* seeds extract: characterization and mechanism of antimicrobial action on different microorganisms. Journal of Analytical Science and Technology 10: 5. https://doi.org/10.1186/s40543-018-0163-z

Saleem U, Riaz S, Ahmad B, Mohammad S (2017) Pharmacological screening of *Trachyspermum ammi* for antihyperlipidemic activity in Triton X-100 induced hyperlipidemia rat model. Pharmacognosy Research 9(1): S34–S40. https://doi.org/10.4103/pr.pr_37_17

Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C (2011) Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitology Research 108: 693–702. https://doi.org/10.1007/s00436-010-2115-4

Sarwer Q, Amjad MS, Mehmood A, Binish Z, Mustafa G, Farooq A, Qaseem MF, Abasi F, Pérez de la Lastra JM (2022) Green synthesis and characterization of silver nanoparticles using *Myrsine africana* leaf extract for their antibacterial, antioxidant and phytotoxic activities. Molecules 29(9): 1922. https://doi.org/10.3390/molecules27217612

Selvaraj R, Pai S, Murugesan G, Pandey S, Bhole R, Gonsalves D, Varadavenkatesan T, Vinayagam R (2021) Green synthesis of magnetic α–Fe2O3 nanospheres using *Bridelia retusa* leaf extract for Fenton-like degradation of crystal violet dye. Applied Nanoscience (Switzerland) 11: 2227–2234. https://doi.org/10.1007/s13204-021-01952-y

Silva LP, Pereira TM, Bonatto CC (2018) Frontiers and perspectives in the green synthesis of silver nanoparticles. In: Green Synthesis, Characterization and Applications of Nanoparticles, 137–164. https://doi.org/10.1016/B978-0-08-102579-6.00007-1

de Souza WM, Ribeiro GS, de Lima STS, de Jesus R, Moreira FRR, Whittaker C, Sallum MAM, Carrington CVF, Sabino EC, Kitron U, Faria NR, Weaver SC (2024) Chikungunya: a decade of burden in the Americas. The Lancet Regional Health – Americas 30: 100673. https://doi.org/10.1016/j.lana.2023.100673

Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Kumar PM, Dinesh D, Chandramohan B, Suresh U, Nicoletti M, Higuchi A, Hwang JS, Kumar S, Alarfaj AA, Munusamy MA, Messing RH, Benelli G (2015) Eco-friendly control of malaria and arbovirus vectors using the mosquitofish *Gambusia affinis* and ultra-low dosages of *Mimusops elengi*-synthesized silver nanoparticles: towards an integrative approach? Environmental Science and Pollution Research 22: 20067–20083. https://doi.org/10.1007/s11356-015-5253-5

Suresh U, Murugan K, Benelli G, Nicoletti M, Barnard DR, Panneerselvam C, Kumar PM, Subramaniam J, Dinesh D, Chandramohan B (2015) Tackling the growing threat of dengue: *Phyllanthus niruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera: Culicidae). Parasitology Research 114: 1551–1562. https://doi.org/10.1007/s00436-015-4339-9

Suriati G, Mariatti M, Azizan A (2014) Synthesis of silver nanoparticles by chemical reduction method: Effect of reducing agent and surfactant concentration. International Journal of Automotive and Mechanical Engineering 10: 1920–1927. https://doi.org/10.15282/ijame.10.2014.9.0160

Veerakumar K, Govindarajan M, Hoti SL (2014) Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes. Parasitology Research 113: 4567–4577. https://doi.org/10.1007/s00436-014-4147-7

Venkatesan P (2024) The 2023 WHO World malaria report. The Lancet Microbe 5(3): E214. https://doi.org/10.1016/S2666-5247(24)00016-8

Wender H, Migowski P, Feil AF, Teixeira SR, Dupont J (2013) Sputtering deposition of nanoparticles onto liquid substrates: Recent advances and future trends. Coordination Chemistry Reviews 257(17–18): 2468–2483. https://doi.org/10.1016/j.ccr.2013.01.013

WHO [World Health Organization] (2005) Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization. https://www.who.int/publications/i/item/WHO-CDS-WHOPES-GCDPP-2005.13

WHO [World Health Organization] (2022) Standard operating procedure for impregnation of filter papers for testing insecticide susceptibility of adult mosquitoes in WHO tube tests. World Health Organization. https://www.who.int/publications/i/item/9789240043817

Zahir AA, Rahuman AA (2012) Evaluation of different extracts and synthesised silver nanoparticles from leaves of *Euphorbia prostrata* against *Haemaphysalis bispinosa* and *Hippobosca maculata*. Veterinary Parasitology 187(3–4): 511–520. https://doi.org/10.1016/j.vetpar.2012.02.001

Zargham F, Afzal M, Rasool K, Manzoor S, Qureshi NA (2023) Larvicidal activity of green synthesized iron oxide nanoparticles using *Grevillea robusta* Cunn. leaf extract against vector mosquitoes and their characterization. Experimental Parasitology 252: 108586. https://doi.org/10.1016/j.exppara.2023.108586

Zulhussnain M, Zahoor MK, Rizvi H, Zahoor MA, Rasul A, Ahmad A, Majeed HN, Rasul A, Ranian K, Jabeen F (2020) Insecticidal and Genotoxic effects of some indigenous plant extracts in *Culex quinquefasciatus* Say Mosquitoes. Scientific Reports 10: 6826. https://doi.org/10.1038/s41598-020-63815-w